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| 10/669,869  | 09/23/2003  | Cyrus Rustam Kumana  | UHK 00091                 | 5138                   |
| 23579 7590 09/21/2007<br>PATREA L. PABST<br>PABST PATENT GROUP LLP<br>400 COLONY SQUARE, SUITE 1200<br>1201 PEACHTREE STREET<br>ATLANTA, GA 30361 |             |                      | EXAMINER<br>CHOI, FRANK I |                        |
|   |             |                      | ART UNIT<br>1616          | PAPER NUMBER           |
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**Please find below and/or attached an Office communication concerning this application or proceeding.**

The time period for reply, if any, is set in the attached communication.

|                          |                                      |                                      |  |
|--------------------------|--------------------------------------|--------------------------------------|--|
| <b>Interview Summary</b> | <b>Application No.</b><br>10/669,869 | <b>Applicant(s)</b><br>KUMANA ET AL. |  |
|                          | <b>Examiner</b><br>Frank I. Choi     | <b>Art Unit</b><br>1616              |  |

All participants (applicant, applicant's representative, PTO personnel):

(1) Frank I. Choi.

(3) Yok-Lam Wong.

(2) Cyrus Kumana.

(4) Eliza Kung; (5) Patrea Pabst.

Date of Interview: 06 September 2007.

Type: a) ☒ Telephonic b) ☐ Video Conference  
c) ☐ Personal [copy given to: 1) ☐ applicant 2) ☐ applicant's representative]

Exhibit shown or demonstration conducted: d) ☐ Yes e) ☒ No.  
If Yes, brief description: \_\_\_\_\_.

Claim(s) discussed: 1 and 28.


Identification of prior art discussed: WO 99/24029, CN1370540.

Agreement with respect to the claims f) ☐ was reached. g) ☒ was not reached. h) ☐ N/A.

Substance of Interview including description of the general nature of what was agreed to if an agreement was reached, or any other comments: See Continuation Sheet.

(A fuller description, if necessary, and a copy of the amendments which the examiner agreed would render the claims allowable, if available, must be attached. Also, where no copy of the amendments that would render the claims allowable is available, a summary thereof must be attached.)

THE FORMAL WRITTEN REPLY TO THE LAST OFFICE ACTION MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW. (See MPEP Section 713.04). If a reply to the last Office action has already been filed, APPLICANT IS GIVEN A NON-EXTENDABLE PERIOD OF THE LONGER OF ONE MONTH OR THIRTY DAYS FROM THIS INTERVIEW DATE, OR THE MAILING DATE OF THIS INTERVIEW SUMMARY FORM, WHICHEVER IS LATER, TO FILE A STATEMENT OF THE SUBSTANCE OF THE INTERVIEW. See Summary of Record of Interview requirements on reverse side or on attached sheet.

  
JOHANN RICHTER  
SUPERVISORY PATENT EXAMINER  
GROUP 1600



Examiner Note: You must sign this form unless it is an Attachment to a signed Office action.

Examiner's signature, if required

## Summary of Record of Interview Requirements

### Manual of Patent Examining Procedure (MPEP), Section 713.04, Substance of Interview Must be Made of Record

A complete written statement as to the substance of any face-to-face, video conference, or telephone interview with regard to an application must be made of record in the application whether or not an agreement with the examiner was reached at the interview.

### Title 37 Code of Federal Regulations (CFR) § 1.133 Interviews Paragraph (b)

In every instance where reconsideration is requested in view of an interview with an examiner, a complete written statement of the reasons presented at the interview as warranting favorable action must be filed by the applicant. An interview does not remove the necessity for reply to Office action as specified in §§ 1.111, 1.135. (35 U.S.C. 132)

#### 37 CFR §1.2 Business to be transacted in writing.

All business with the Patent or Trademark Office should be transacted in writing. The personal attendance of applicants or their attorneys or agents at the Patent and Trademark Office is unnecessary. The action of the Patent and Trademark Office will be based exclusively on the written record in the Office. No attention will be paid to any alleged oral promise, stipulation, or understanding in relation to which there is disagreement or doubt.

The action of the Patent and Trademark Office cannot be based exclusively on the written record in the Office if that record is itself incomplete through the failure to record the substance of interviews.

It is the responsibility of the applicant or the attorney or agent to make the substance of an interview of record in the application file, unless the examiner indicates he or she will do so. It is the examiner's responsibility to see that such a record is made and to correct material inaccuracies which bear directly on the question of patentability.

Examiners must complete an Interview Summary Form for each interview held where a matter of substance has been discussed during the interview by checking the appropriate boxes and filling in the blanks. Discussions regarding only procedural matters, directed solely to restriction requirements for which interview recordation is otherwise provided for in Section 812.01 of the Manual of Patent Examining Procedure, or pointing out typographical errors or unreadable script in Office actions or the like, are excluded from the interview recordation procedures below. Where the substance of an interview is completely recorded in an Examiners Amendment, no separate Interview Summary Record is required.

The Interview Summary Form shall be given an appropriate Paper No., placed in the right hand portion of the file, and listed on the "Contents" section of the file wrapper. In a personal interview, a duplicate of the Form is given to the applicant (or attorney or agent) at the conclusion of the interview. In the case of a telephone or video-conference interview, the copy is mailed to the applicant's correspondence address either with or prior to the next official communication. If additional correspondence from the examiner is not likely before an allowance or if other circumstances dictate, the Form should be mailed promptly after the interview rather than with the next official communication.

The Form provides for recordation of the following information:

- Application Number (Series Code and Serial Number)
- Name of applicant
- Name of examiner
- Date of interview
- Type of interview (telephonic, video-conference, or personal)
- Name of participant(s) (applicant, attorney or agent, examiner, other PTO personnel, etc.)
- An indication whether or not an exhibit was shown or a demonstration conducted
- An identification of the specific prior art discussed
- An indication whether an agreement was reached and if so, a description of the general nature of the agreement (may be by attachment of a copy of amendments or claims agreed as being allowable). Note: Agreement as to allowability is tentative and does not restrict further action by the examiner to the contrary.
- The signature of the examiner who conducted the interview (if Form is not an attachment to a signed Office action)

It is desirable that the examiner orally remind the applicant of his or her obligation to record the substance of the interview of each case. It should be noted, however, that the Interview Summary Form will not normally be considered a complete and proper recordation of the interview unless it includes, or is supplemented by the applicant or the examiner to include, all of the applicable items required below concerning the substance of the interview.

A complete and proper recordation of the substance of any interview should include at least the following applicable items:

- 1) A brief description of the nature of any exhibit shown or any demonstration conducted,
- 2) an identification of the claims discussed,
- 3) an identification of the specific prior art discussed,
- 4) an identification of the principal proposed amendments of a substantive nature discussed, unless these are already described on the Interview Summary Form completed by the Examiner,
- 5) a brief identification of the general thrust of the principal arguments presented to the examiner,  
(The identification of arguments need not be lengthy or elaborate. A verbatim or highly detailed description of the arguments is not required. The identification of the arguments is sufficient if the general nature or thrust of the principal arguments made to the examiner can be understood in the context of the application file. Of course, the applicant may desire to emphasize and fully describe those arguments which he or she feels were or might be persuasive to the examiner.)
- 6) a general indication of any other pertinent matters discussed, and
- 7) if appropriate, the general results or outcome of the interview unless already described in the Interview Summary Form completed by the examiner.

Examiners are expected to carefully review the applicant's record of the substance of an interview. If the record is not complete and accurate, the examiner will give the applicant an extendable one month time period to correct the record.

### Examiner to Check for Accuracy

If the claims are allowable for other reasons of record, the examiner should send a letter setting forth the examiner's version of the statement attributed to him or her. If the record is complete and accurate, the examiner should place the indication, "Interview Record OK" on the paper recording the substance of the interview along with the date and the examiner's initials.

Continuation of Substance of Interview including description of the general nature of what was agreed to if an agreement was reached, or any other comments: Written description rejection was discussed. The examiner indicated that would consider amendment to method claim specifically indicating that the side effect at issue was cardiac arrhythmia for purposes of written description issue and 103 rejection, but that support for composition limitation relative to the amount being less than the effective amount for intravenous administration did not appear to have support. Examiner indicated that with respect to the 102 rejections relative to the compositions that the issue was not whether the inventor knows of the use of the compositions orally; instead, the Applicant must show that the compositions cannot be used orally.

different concentrations. Consistent with our earlier report, incubation of Karpas 299 cells with immobilized antibodies to CD30 was found to potently induce cell death, whereas in the same experiment the addition of these antibodies in their soluble form did not induce cell death and actually slightly enhanced viability (Figure 1). Also noteworthy is the fact that, in the earlier report by Gruss et al<sup>4</sup> in which the cytotoxic effects of M44 and M67 were originally described, plate-bound antibodies were used. In preliminary experiments using plate-bound HeFi-1, we have also observed a proapoptotic effect (data not shown).

In summary, we suggest that apparently different experimental methods are the most likely explanation for the discrepancies between our report and that of Levi et al, in particular the use of immobilized antibody versus plate-bound antibody. The different effects of soluble and immobilized antibody are suggestive of an intriguing physiologic mechanism by which low levels of CD30 activation may induce cell-cycle arrest, activation of NF- $\kappa$ B, and the concomitant induction of antiapoptotic genes, whereas a stronger CD30-activation signal may result in a transient and more limited activation of NF- $\kappa$ B and ultimately cell death. The strength of the CD30 signal may be determined by numerous factors, including the density of CD30 receptors on the cell and the form of ligand (ie, membrane-bound or soluble). The threshold sensitivity of a given cell may also be determined by additional factors, such as the stability of intracellular

signaling intermediates, particularly TRAF2. Thus, the apparent discrepancies between our data and those of Levi et al may reflect a novel physiologic function of CD30.

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3. Mir SS, Richter BWM, Duckett CS. Differential effects of CD30 activation in anaplastic large cell lymphoma and Hodgkin Disease cells. *Blood*. 2000;15:4307-4312.
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## To the editor

### Cardiac toxicity of arsenic trioxide

Unnikrishnan et al have reported the occurrence of torsades de pointes in 3 of 19 patients treated with arsenic trioxide.<sup>1</sup> The propensity for arsenic to cause reversible Q-T interval prolongation is well known and has previously been characterized.<sup>2</sup> To minimize the risk of long Q-T-related arrhythmias associated with arsenic therapy, Cell Therapeutics, makers of the FDA-approved Trisenox (arsenic trioxide), have issued very specific guidelines regarding electrolyte monitoring and replacement, as well as avoidance of concomitant Q-T interval prolonging in patients being treated with their compound.<sup>3</sup>

Three specific points regarding the cases submitted by Unnikrishnan et al are worth emphasizing. The rhythm strip submitted for patient 2 clearly demonstrates the "long-short" initiation sequence pathognomonic for torsades de pointes. Presumably, strips from patients 1 and 3 display similar initiating sequences thus supporting a diagnosis of acquired long Q-T syndrome rather than that of nonspecific cardiac arrest, a distinction not always easy to make in such sick patients. All 3 patients displayed widely fluctuating serum levels of potassium and magnesium. Hypokalemia, hypomagnesemia, and diuretic use clearly increase the risk of long Q-T-related arrhythmias, making aggressive electrolyte replacement mandatory in such a population. Finally, the Q-Tc intervals reported in Unnikrishnan et al's Table 1 appear surprisingly short for patients experiencing torsades de pointes. These values are likely machine generated and are probably incorrect in view of the marked tachycardia and T-wave flattening observed in these patients.

In summary, arsenic trioxide prolongs the Q-T interval in a gradual and reversible fashion and is liable to cause torsade de pointes unless stringent precautions are taken. Aggressive electrolyte replacement is mandatory, and monitoring of the Q-T interval warranted, recognizing that machine generated values may become unreliable in the presence of marked tachycardia and T-wave flattening. Finally, if cardiac arrhythmias occur, distinguishing torsades de pointes (an adverse drug reaction until proven otherwise) from other arrhythmias has important therapeutic and public health implications.

Jean T. Barbey

Correspondence: Division of Clinical Pharmacology, Georgetown University, Washington DC

J.T.B. was a paid consultant for Cell Therapeutics but currently has no financial interest in that company or in any competing company.

## References

1. Unnikrishnan D, Dutcher JP, Varsheneya N, et al. Torsades de Pointes in 3 patients with leukemia treated with arsenic trioxide. *Blood*. 2001;97:1514-1516.
2. Barbey JT, Soignet SL. Arsenic trioxide (ATO): analysis of QT prolongation on electrocardiogram (ECG) [abstract]. *Blood*. 2000;96:319a.
3. Trisenox [package insert]. Seattle, WA: Cell Therapeutics; 2000.

## To the editor:

### Cardiac toxicity of arsenic trioxide

Unnikrishnan et al describe 3 patients out of 19 treated with arsenic trioxide who developed torsades de pointes (TDP) that proved fatal in 2. The source of the arsenic trioxide given these patients and its characterization and formulation are not stated in the article. But inasmuch as the article was submitted to *Blood* prior to FDA approval of Trisenox brand of arsenic trioxide for injection on September 25, 2000, and no Trisenox studies under the Cell Therapeutics investigational new drug application (IND) were performed at Our Lady of Mercy Cancer Center, we are sure that the arsenic trioxide formulation administered in that study was not manufactured by Cell Therapeutics, the only commercial source of this agent in the United States.

We believe this distinction is important because the safety experience reported for Trisenox has been very different from that reported in the Unnikrishnan et al article and that reported with another research product by Westervelt et al,<sup>2</sup> a report cited in Unnikrishnan et al. As director of the Pharmacovigilance Committee at Cell Therapeutics, I would like to review our safety experience to date with Trisenox, particularly with reference to Q-T prolongation, TDP, and the FDA-approved guidelines for safe and effective use of Trisenox as described in the product label.

Trisenox for injection was approved for marketing in September 2000, for the treatment of relapsed and refractory acute promyelocytic leukemia (APL). In the multicenter pivotal study of Trisenox in relapsed or refractory APL, 70% of the patients achieved a complete remission, with 78% demonstrating a molecular remission. The median survival is greater than 18 months.

Our current pharmacovigilance database consists of more than 360 patients treated with Trisenox at doses ranging from 0.15 to 0.35 mg/kg/d. These data include patients treated on clinical investigations in a variety of malignancies conducted under our company-sponsored IND, in NCI-sponsored trials under a Cooperative Research and Development Agreement (CRADA), or on a compassionate-use program for patients with APL. In addition, postmarketing surveillance adverse-event reports on patients treated since product launch in October 2000 were evaluated. Q-T prolongation is a well known and expected effect of arsenic trioxide treatment. To date, only 3 cases of Q-T prolongation above 500 millisecond (ms) have been reported. No deaths due to cardiac arrhythmias have been attributed to Trisenox.

In a detailed independent review of 1000 electrocardiograms from 99 patients on clinical trials, 26 cases of Q-Tc above 500 ms were identified, with 3 cases having an absolute Q-T above 500 ms. A single case of self-limited TDP occurred in a patient undergoing

induction therapy for APL who was also receiving amphotericin B. The TDP resolved after electrolyte correction, and the patient went on to receive consolidation therapy uneventfully. Under the management guidelines included in the label, treatment emergent adverse events have decreased over time, are less common in consolidation and maintenance, and usually have not required stopping therapy.

The high frequency and severity of complex arrhythmias or deaths reported by Unnikrishnan et al and Westervelt et al, 2 sites using investigational arsenic trioxide, appear to be different from the relatively lower frequency of events that have been reported with Trisenox. Why these experiences differ so markedly from that reported by Unnikrishnan et al or Westervelt et al is uncertain. It should be noted that patients 1 and 2 in the Unnikrishnan et al study received 20 mg of arsenic trioxide per day, potentially a higher than recommended dose of 0.15 mg/kg/d, although the patient weights were not given. Both patients had marked hypomagnesemia, hypokalemia, and pulmonary failure at the time they developed TDP. The "black box" warning in the Trisenox label recommends that the potassium and magnesium values be maintained at midnormal levels when administering Trisenox (at least 4 mEq/L for potassium and 1.8 mg/dL for magnesium). The third patient received 10 mg/d of arsenic trioxide, which was stopped after 7 days for prolongation of the Q-Tc. TDP developed after the drug had been discontinued for 5 days and the Q-T interval was normalizing. As this patient also had respiratory failure and was on a ventilator, factors other than arsenic trioxide may have contributed to his refractory arrhythmia.

Based on the available data on more than 360 patients, the safety of administration of Trisenox can be optimized with appropriate monitoring and management of electrocardiogram abnormalities as described in the product label.

Jack W. Singer

Correspondence: Cell Therapeutics, 201 Elliot Ave, West, Suite 400, Seattle, WA 98119

J.W.S. is Executive Vice President of Cell Therapeutics and holds more than \$10,000 worth of stock in that company.

### References

- Unnikrishnan D, Dutcher JP, Varshneya N, et al. Torsades de Pointes in 3 patients with leukemia treated with arsenic trioxide. *Blood*. 2001;97:1514-1516.
- Westervelt P, Brown R, Adkins D, et al. Sudden deaths among acute promyelocytic leukemia patients treated with arsenic trioxide [abstract]. *Blood*. 2000;96:723a.

## Response:

### Monitoring of cardiac toxicity with arsenic trioxide

We thank you for the opportunity to comment on the letters by Barbey and by Singer, regarding our brief report on the occurrence of torsades de pointes in 3 patients with relapsed/refractory acute myelogenous leukemia treated with arsenic trioxide. First, in response to Dr Barbey, the arrhythmias observed in all 3 patients were preceded by the long-short initiation sequence. We did not have room to present electrocardiograms (ECGs) from all 3 patients. In 2 of the patients, this rhythm was not symptomatic initially but did recur. It was not the cause of death in these 2 patients. In discussion of the electrolytes, "widely fluctuating" is a

bit of an overstatement. Nevertheless, it is clear that the variation in the values presented in the manuscript are consistent with observations obtained in seriously ill, hospitalized patients with acute leukemia, who require daily monitoring of blood values, including electrolytes, and who require frequent replacement of electrolytes. These patients are not simple to manage, in that they require multiple antibiotics and blood products, in addition to daily arsenic, and intravenous intake frequently exceeds 3 liters per day in such patients. Additionally, the fluid retention syndrome noted with the use of arsenic trioxide makes fluid and electrolyte balance even

more difficult, and diuretics may be necessary. In contrast, acute promyelocytic leukemia patients, in whom molecular or cytogenetic relapse can be detected prior to florid blastic leukemia, may not have such severe problems and may be more easily managed. But Q-Tc prolongation and ventricular arrhythmias have been observed even in this population.<sup>1</sup>

The third point by Dr Barbey is not correct. The Q-Tc values presented were not machine values, but values calculated independently by 2 cardiologists and then confirmed. We agree that the degree of prolongation was moderate, but the observation that we made that may be more important than an isolated Q-Tc interval was our observation of serial prolongation of Q-Tc, occurring in these patients as treatment progressed. Thus, the reliability of an isolated prolonged Q-Tc may be less valuable than serial measurements in predicting the risk of arrhythmia.

With respect to the comments by Dr Singer, our initial point in the brief report was to demonstrate the multiple factors that impact on seriously ill leukemia patients, in addition to the therapy with arsenic trioxide. But since torsades de pointes is known to be associated with arsenic ingestion, as is Q-Tc prolongation, careful and serial monitoring is required.<sup>1-4</sup> We agree with the recommen-

dations provided in the package insert, and we concur that careful attention to electrolyte replacement is important. Whether that is sufficient remains to be determined, and further evaluation of the electrophysiology seems warranted.

Dilip Unnikrishnan, Janice P. Dutcher, Nikita Varshneya, Richard Lucarello, Peter H. Wiemik, and Salvatore Chiamaramida

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## References

- Ohnishi K, Yoshida H, Shigeno K, et al. Prolongation of the QT interval and ventricular tachycardia in patients treated with arsenic trioxide for acute promyelocytic leukemia. *Ann Intern Med*. 2000;133:881-885.
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- Westervelt P, Brown RA, Adkins DR, et al. Sudden death among patients with acute promyelocytic leukemia treated with arsenic trioxide. *Blood*. 2001;98:266-271.

## To the editor:

### Meta-analysis of the association between low-affinity *Fcγ* receptor gene polymorphisms and hematologic and autoimmune diseases

Genetic polymorphisms of the low-affinity receptors of the Fc domain of IgG (*FcγR*) have been proposed to be associated with an array of hematologic, autoimmune, and other diseases. Most studies have addressed the *R/H131* polymorphism of the *FcγRIIIa* isoform<sup>1</sup> and the *V/F158* polymorphism of the *FcγRIIIa* isoform.<sup>2</sup> For the majority of the associations, only single studies have been performed. But for conditions such as heparin-induced thrombocytopenia, systemic lupus erythematosus (SLE) and SLE-related nephritis, and idiopathic thrombocytopenic purpura, data have been generated from multiple teams of investigators. Information from different teams has often been controversial or even conflicting. We believe that there is a need to standardize and synthesize the accumulating data across these various studies.

Lehrnbecher et al<sup>3</sup> made an effort to accumulate in a systematic fashion the epidemiologic data on genetic associations involving low-affinity *FcγR* receptor polymorphisms. Unfortunately, the synthesis of the data was problematic. Typically, when there was more than one study addressing the same association, the number of subjects in the patient and control arms were summed for the various genotypes and statistical tests were performed in the resulting contingency tables with the summed data. This approach is methodologically inappropriate. The synthesis of data across diverse studies needs to take into account the within study variance, and when there is detectable statistical heterogeneity in the measure of association between the studies, it would be prudent to also take into account an estimate of the variance between the studies.<sup>4</sup> Such a formal meta-analytic approach may yield very different results compared to simple summation.

As an example, the figure shows a meta-analysis for the association of the *FcγRIIIa H/H131* genotype with heparin-induced thrombocytopenia, analyzing the same databases as in the Lehrnbecher et al paper, which had suggested a possible negative

association of *H/H131* with heparin-induced thrombocytopenia ( $P = .13$ ). To simply sum up the data from 6 studies, *H/H131* is found in 151 of the 626 patients (24.1%) versus in 359 of the 1313 controls (27.3%). The crude odds ratio is 0.84 (95% CI, 0.68-1.05). In truth, here is a situation in which there is highly statistically significant heterogeneity between the 6 studies ( $P < .001$  for heterogeneity based on the Q statistic).<sup>4</sup> As shown in Figure 1, some studies suggest a strongly positive association, while others suggest a negative association. Simple summation is misleading. By random effects calculations,<sup>5</sup> the summary odds ratio is 1.11 (95% CI, 0.56-2.19) and the  $P$  value for the association is .77, which suggests absolutely no consistent effect.

The table shows the summary odds ratios by fixed and

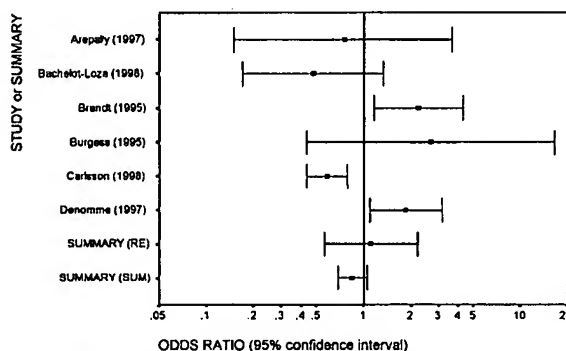


Figure 1. Meta-analysis of 6 studies addressing the association of *FcγRIIIa R131H* polymorphism with heparin-induced thrombocytopenia. Each study is shown as a point-estimate odds ratio, with lines extending to the 95% confidence interval of the odds ratio. The summary effect is obtained with random effects (RE) calculations, and for comparison we also show a summary effect based on simply adding up the numbers across studies (SUM). The numerical data are presented in Lehrnbecher et al.<sup>3</sup>

our RT-PCR protocol enabled us to detect *WT1* gene expression in the same percentage of acute leukemia MNC preparations as compared to others.<sup>3,4</sup> Since more sensitive RT-PCR protocols detect low *WT1* gene expression levels in normal blood and bone marrow (BM) MNCs, quantitative RT-PCR had to be implemented to discriminate between a physiologic and a malignant, leukemia-associated expression level of this gene.<sup>3</sup> Contrary to acute leukemia, we never detected the *WT1* nuclear protein in MNC preparations from normal blood and BM, or from leukapheresis products of solid cancer patients, using a single cell indirect immunofluorescence assay with anti-*WT1* monoclonal antibodies.<sup>8</sup> Thus, it remains unclear, whether the detection of low-level *WT1* gene expression in normal blood cells and hematopoietic progenitors by highly-sensitive RT-PCR protocols reflects "illegitimate or ectopic transcripts" or may have a physiologic significance. To our surprise, we found *WT1* gene transcripts in almost all hematopoietic soft agar colonies at day 14 but not thereafter, although single colonies at day 14 contain only 100 to 300 as compared to 800 to 1,000 cells at day 28, indicating transient *WT1* gene expression in hematopoietic progenitor cells during their early exponential growth.

Finally, we hypothesize that expression of the *WT1* gene is relevant to the fetal development and physiologic expansion of immature CD34<sup>+</sup> hematopoietic progenitors, and that the *WT1* gene is functionally switched off on their determination and differentiation. This hypothesis explains acute leukemia as a proliferative disorder, which is at least partly arrested in a state of *WT1* gene-expressing stem cell expansion. It further explains, why the *WT1* gene is downregulated in differentiation-induced leukemia cell lines, why antisense-*WT1* oligonucleotides reduce growth of acute leukemia cell lines, and why subsets of normal regenerating BM CD34<sup>+</sup> hematopoietic progenitors express the *WT1* gene on levels comparable to leukemia blasts.<sup>6</sup>

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### Delicious Poison: Arsenic Trioxide for the Treatment of Leukemia

To the Editor:

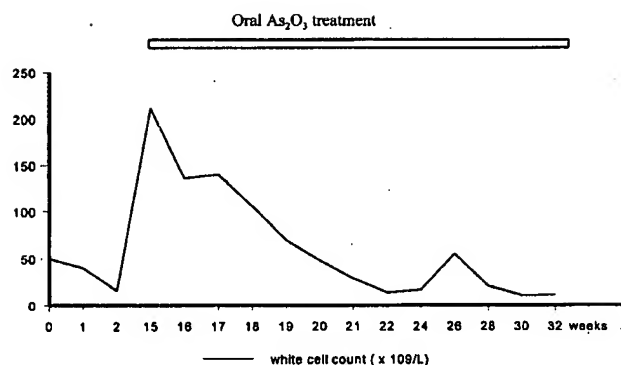
Lately, arsenic trioxide ( $\text{As}_2\text{O}_3$ ) has been described in the treatment of acute myeloid leukemia. Experiments *in vitro* showed that  $\text{As}_2\text{O}_3$  induced the acute promyelocytic leukemia (APL) cell line NB4 to downregulate *bcl-2* expression, as well as to undergo apoptosis.<sup>1</sup> Clinically efficacy has been shown in 14 of 15 patients with relapsed APL, where the use of intravenous  $\text{As}_2\text{O}_3$  at a dose of 10 mg/d for 4 to 9 weeks resulted in complete morphologic remission without associated bone marrow suppression.<sup>2</sup> In these cases, partial differentiation of the APL cells and downregulation of the fusion protein PML/RAR $\alpha$  could also be shown, which might account for the pharmacologic action of the drug.<sup>3</sup>

Arsenic has been known to be poisonous for centuries. Medicinal use of arsenic began in the 15th century. In the 18th century, Dr Thomas Fowler developed a solution preparation of  $\text{As}_2\text{O}_3$  in potassium bicarbonate (1% w/vol), known generally as Fowler's solution,<sup>4</sup> which was used empirically for the treatment of a variety of infectious and malignant diseases. The effect of Fowler's solution on the reduction of white cells in two normal people and one patient with "leucocythemia" studied at Boston City Hospital, MA was first described in 1878.<sup>5</sup> This led to the use of  $\text{As}_2\text{O}_3$  for the treatment of

leukemia, until the advent of radiotherapy caused a decline in its clinical application. Its popularity waxed again when Forkner and Scott,<sup>6</sup> also at Boston City Hospital, described nine of 10 patients with chronic myeloid leukemia (CML) who responded to  $\text{As}_2\text{O}_3$  treatment. These results were subsequently confirmed by other reports,<sup>7</sup> so that  $\text{As}_2\text{O}_3$  was considered next to irradiation as the most effective treatment of CML before the development of modern chemotherapy.<sup>8</sup> Clinical improvement of the leukemia, including the control of fever, reduction of white cell count, amelioration of anemia and decrease in the size of spleen, could often be achieved. Sometimes, a remission might be maintained for a long period. As expected, toxic side effects were observed in the majority of patients given long-term  $\text{As}_2\text{O}_3$ , including skin pigmentation and keratosis, cirrhosis, polyneuritis, and gastrointestinal problems.<sup>9</sup> In this department,  $\text{As}_2\text{O}_3$  was used by hematologists in the 1950's for the treatment of a variety of leukemias. Figure 1 shows the typical course of a patient treated with  $\text{As}_2\text{O}_3$  for CML in chronic phase. As  $\text{As}_2\text{O}_3$  appeared to be effective for leukemias of different morphologic types, the action was probably related to an intrinsic toxicity of arsenic to marrow cells.

Therefore, while  $\text{As}_2\text{O}_3$  induced apoptosis and differentiation of APL cells is a novel observation, its clinical use represents but a





**Fig 1.** A 30-year-old man presented in March 1954 with splenomegaly and CML in chronic phase was diagnosed. No specific treatment was given until October 1954 when his splenomegaly increased to 5 cm and his white cell count increased to  $50 \times 10^9/L$ . Fowler's solution 5 minims (1 minim = 0.06 mL, equivalent to 0.6 mg  $As_2O_3$ ) three times daily was administered, resulting in a satisfactory control of his white cell count to about  $10 \times 10^9/L$ . Treatment was stopped. Six months later, he was readmitted with progressive splenomegaly (10 cm) and leucocytosis ( $211 \times 10^9/L$ ). Fowler's solution was recommenced at 5 minims three times daily, and increased to 10 minims three times daily. This resulted in gradual control of his white cell count. The dose of  $As_2O_3$  was decreased to a maintenance dose of 5 minims three times daily. However, 8 months later, signs and symptoms of chronic arsenic poisoning developed, including skin pigmentation, diarrhea, and chronic gastrointestinal hemorrhage.  $As_2O_3$  was stopped and he was put on melphalan. Splenomegaly and leucocytosis progressed despite treatment, and he died 11 months later of pneumonia. The maximum daily dose (10 minims  $\times$  3) of  $As_2O_3$  given orally was 18 mg, which is comparable to 10 mg/d when used intravenously for the treatment of relapsed APL.

resurgence of the use of arsenicals in the treatment of leukemia. Because of the considerable toxicities and the possible and still undefined long-term sequelae, the usefulness of  $As_2O_3$  in the modern treatment of leukemia is still unclear. For this reason, Fig 1 is only of historical interest. However, the use of heavy metals in the treatment of malignancies is not unprecedented, with platinum being a prominent example. Therefore, the rekindling of the interest in arsenicals is of potential importance, but the biological and pharmacologi-

cal actions of arsenic must be further investigated to define its role in the treatment of leukemia and other types of malignancies.

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#### Evidence That the Expression and Phosphorylation Status of Pleckstrin Is Modulated by Epstein-Barr Virus in Human B Lymphocytes

To the Editor:

Pleckstrin is exclusively expressed in human hemopoietic cells and is induced during differentiation. Pleckstrin protein contains two copies of the prototypic pleckstrin homology (PH) domain and has been shown to be hyperphosphorylated and appears to have a role in signal transduction.<sup>1</sup> Recently we reported that the coordinate expression of the Epstein-Barr virus (EBV) encoded EBNA3, 4 and 6 proteins (EBNA 3 family) lead to the upregulation of pleckstrin protein in the transfected Burkitt's lymphoma (BL) cell line dG75.<sup>2</sup> The present study was undertaken to determine if pleckstrin was upregulated in EBV<sup>+</sup> cells and to evaluate its phosphorylation status.

Isogenic cell pairs of BL cells or the corresponding lymphoblastoid cell line expressing either the EBNA1 protein alone (Mutu-I, BL29) or the full set of EBV latency proteins (Mutu-III, IARC167)

were used. The cell phenotype and the expression of EBV genes were analyzed by fluorescence-activated cell sorter and immunoblot analysis. Equal numbers of exponentially growing cells were radiolabeled with <sup>32</sup>P and the cell lysates subjected to immunoprecipitation using rabbit antipleckstrin serum<sup>1</sup> followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Fig 1A). To determine the amount of total pleckstrin protein in the radiolabeled cells, aliquots of the cell lysates were analyzed in parallel by immunoblot using the rabbit antipleckstrin serum (Fig 1B). Analysis of the polyacrylamide gels by silverstaining and of the nitrocellulose filters by staining with Ponceaus (Sigma, Castle Hill, NSW, Australia) and with an anti- $\beta$ II microglobulin antibody confirmed that each sample contained similar amounts of protein.

Pleckstrin protein was highly expressed in each of the cell lines expressing the EBV-latency antigens (Fig 1B, lanes 2 and 4). How-